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EFFECT OF PHYTOBIOTICS AND ANTIBIOTIC ON GROWTH PERFORMANCE, INTESTINAL MORPHOLOGY AND NUTRIENTS TRANSPORTERS EXPRESSION OF BROILER CHICKENS

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ABSTRACT: *This study investigated the effect of Phytobiotics (PB) and Antibiotic Growth Promoter (AGP) on performance, intestinal morphology and nutrients transporters mRNA gene expression of broiler chickens. One hundred eighty unsexed one-day-old Ross strains with an average initial body weight (BW) of 45 ± 0.5 g were randomly assigned into three treatments with six replications of 10 chicks in each pen. (1) Control group (CON), basal diets only; (2) antibiotic group, Antibiotic Growth promoter (AGP), basal diet supplemented with 5 mg/kg tetramycin; (3) Phytobiotics (PB), basal diet supplemented with 0.5g/kg, phytobiotics the research lasted for 42 days. Average Daily Feed Intake (ADFI), Average Daily Gain (ADG) and Feed Conversion Ratio (FCR) were calculated weekly, and intestinal morphology of Villus Height (VH), Crypt Depth (CD), and Villus Height to crypt depth ratio (VH: CD) from the duodenum, jejunum, and ileum, nutrients transporters qRT-PCR mRNA expression of GLUT1, SGLT4, GLUT5, PEPT2, CAT1, and LAT1 were evaluated at day 42. Chicks fed PB had significantly (P=0.0413) higher ADG at 1-42 days, PB and AGP significantly had lower FCR (P=0.0001) and (P=0.0001) at 22-42 d and 1-42 days, respectively, in duodenum the villus height was significantly (P=0.0001) longer in PB fed chickens and the lowest was recorded in CON, while the CD and VH: CD were significantly longer in AGP and PB fed chickens, in jejunum VH and CD were significant (P=0.0001) longer in PB and AGP than the CON fed chickens, in ileum PB fed birds had significant higher VH and CD than the AGP and CON fed groups, in duodenum and ileum GLUT1, SGLT4 and GLUT5 expression were highly expressed in AGP and PB fed chickens similar results was obtained in jejunum and ileum by PEPT2 and CAT1. These findings suggest that supplementing phytobiotics in broiler diets improves growth performance and intestinal morphology and up-regulated the expression of nutrient transporters' genes. However, the underlying detailed biological mechanisms and dose standardisation for inclusion in broiler diets need to be further studied.*

KEYWORDS: Broiler, Phytobiotics, AGP, Body Weight gain, FCR, Nutrients Transporters.

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INTRODUCTION

The poultry industry is currently considered as one of the fastest-growing subsector of agriculture due to the increased consumption of meat and egg. The industry serves to fill the gaps between the requirement and availability of quality protein rich in most essential nutrients for human consumption (Dhama et al., 2015). The industry has grown so fast alongside the use of antibiotics to maximise profit and make production cost-effective during the last few decades. Antimicrobial growth promoters (AGP) have been widely used as an effective tool in livestock and poultry to boost protection against pathogens and productivity for decades, Brown et al., (2017).

The rising concern about the indiscriminate use of these antibiotics has resulted in the deposition of residues in poultry food products and the development of resistance to these drugs by microorganisms. Therefore, many diseases are becoming difficult to treat in humans and animals. In the same way, scientific evidence suggests a link between antibiotic resistance and its potentially harmful effect on the intestinal microbiota of poultry. Boeckel et al. 2015; Haque et al., 2020). Thus, the use of antimicrobials in food animal production became worrisome, and these situations forced the international community to restrict the utilisation of antibiotic growth promoters in feed animals (Hady et al., 2016). This restriction resulted in numerous problems, such as the prevalence of enteric diseases, an increase in feed conversion ratio (FCR), and the incidence and outbreak of animal diseases, among others. (Anadón et al., 2018; Kostadinović et al., 2018). In this regard, poultry farmers face challenges in meeting the consumers' demand without the use of antibiotics and improving production (Haque et al., 2020) in their effort to find substitutes to antibiotics growth promoters that can maintain animal productivity and guarantee safety products to consumers, the use of compounds such as phytobiotics, probiotics, prebiotics, symbiotic, organic acids, enzymes, herbs spirulina, para-probiotics among others are being used more frequently in poultry production as substitutes source for antibiotics (Gadde et al., 2017; Musa Bodinga et al., 2020).

Phytobiotics or phytogenics are “plant-derived compounds incorporated into the diets of livestock to improve their productivity through redeeming the feed properties as well as to enhance the quality of food derived by those animals” (Windisch et al., 2008). Phytobiotics are gaining priority as a possible alternative to AGP in poultry production because of their high content of pharmacologically active compounds. They are natural, less toxic, and readily available and do not produce residual effects (Gregacevic et al., 2014; Abd El-Hack et al., 2020a&b). Several studies have reported the positive impacts of phytobiotics in broiler chickens as a possible alternative to AGPs (Wade et al., 2018; Parade et al., 2019), yet their detailed mechanism of action has not yet been completely elucidated (Motoi, 2021). In Nigeria, this type of work is limited. This study aimed to determine the effect of phytogenics on the growth performance, intestinal morphology and nutrients transport efficiency of broiler birds.

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MATERIAL AND METHODS

Ethical Statement

All experimental protocols used in this study were approved by the institutional Animal Care and use committee of Shehu Shagari College of Education, Sokoto State, Nigeria's institutional Animal Care and use committee.

Location of the experimental pen

The experiment was conducted at the poultry unit of the Teaching and Research Farm of the Department of Agricultural Science Shehu Shagari College of Education, Sokoto, Sokoto located in North-West, Nigeria, between longitude 3^0 and 7^0 E and latitude 10^0 and 14^0 N of the equator with latitude and longitude coordinates of 13.005873,5.247552. The Broilers were raised in a deep litter system.

Birds and Experimental Design

One-day-old broilers Ross Strains (n = 180, mixed gender) with an average initial body weight (BW) of 45 ± 0.5 g were acquired from a commercial hatchery, Chikun Nigeria Limited chicks were randomly allotted to one of the three experimental treatments (each treatment group had six cages of ten birds (1) control group (CON, containing basal diets only, (2) antibiotic group (Antibiotic Growth promoter (AGP) group, a basal diet supplemented with 5 mg/kg tetramycin, (3) phytobiotics (PB) group, a basal diet supplemented with 0.5g/kg, Nbiotic™, which is a proprietary polyherbal formulation developed by AYURVET LIMITED, INDIA containing a blended of essential oils and other secondary plant metabolites, the formulation is a herb comprising of *Zingiber officinale*, *Allium sativum*, *Cichorium intybus*, *Eruca sativa*, *Eucalaptus globulus*, *Trigonella foenum-graecum* and manan Oliga saccharide as the main constituents. Birds were fed with a starter diet from 1 to 21 days of age and a finisher diet from 22 to 42 days of age. The composition and nutrient level of the basal diets to meet the Nutritional requirements as recommended by the National Research Council (NRC, 1994) are listed in (Table 1). Feed and freshwater were offered ad libitum with medications as recommended.

Table 1. Composition and nutrients levels of the basal diet (as fed basis)

Ingredient	Starter (%) (day 1-21)	Finisher (%) (day22-42)
Corn	50.90	55.90
Soybean meal (44%)	28.00	23.82
Cotton seed meal	3.00	2.00
Groundnut meal	3.50	2.00
Wheat offal	5.00	5.00
Vegetable oil	2.00	3.50
Tallow	1.53	2.50
Calcium Powder	2.50	2.80
Phosphate (Powder)	1.79	0.15
Lysine sulphate	0.83	0.83
DL-Methionine	0.31	0.32
Sodium Chloride	0.25	0.25

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Tryptophan	0.32	0.28
Threonine	0.18	0.24
Premix[a]	0.24	0.26
Choline Chloride	0.15	0.15
Total	100	100
Chemical composition		
Metabolizable energy (Kcal/kg)	2,930	3,050
Crude protein (CP) %	21.5	19.0
Calcium %	0.95	0.85
Phosphorus %	0.80	0.50
Lysine %	1.12	1.12
Methionine %	0.60	0.61

[a] The premix supplied per kilogram of diet: Vit. A, 15000 IU, Vit. B_1 40mg, Vit B_2 0.80mg, Vit. B_6 0.60mg, Vit. D_3 6,560, IU, Vit. E, 500 IU, Vit. $K_3$30mg, Vt. B_3 5.24g, Zn 20mg, Mn 60mg, Fe, 70mg, Cu 10mg, Mg 0.5mg, Folic acid 25mg,

Sample Collection, measurements and analysis

Growth Performance

Experimental birds were weighed on day one and then on a weekly basis throughout the experimental period to determine the Average Daily gain (ADG). Average Daily Feed intake (ADFI) and weekly feed conversion ratio (FCR) were calculated for the periods of 1 to 21 days, 22 to 42 days, and day 1 to 42 days.

Intestinal histomorphology

Tissue samples from small intestine (duodenum, jejunum and ileum sections were at the following locations (i) the middle part of the duodenal loop, (ii) midpoint between the endpoint of the duodenal loop and Meckel's diverticulum (jejunum) and (iii) midway between the Meckel's diverticulum and the ileo-caecal junction (Choe et al., 2012). The ingesta in the lumen was washed away using normal saline and fixed in 10% buffered formalin. The intestinal portion was conventionally dehydrated in an increasing ethanol series and embedded in paraffin wax. Then, samples were sectioned (5 µm) and stained in haematoxylin–eosin as previously described by Thanh et al. (2009). A computer morphometric program, Optika Vision Pro 3.0, was used for morphometric measurement of the villi height and crypt depth. The villus height was measured from the tip to the base of the villus, and crypt depth was measured from the base of the villus to the mucosa. Averaged height and depth measurements of the 15 randomly selected chickens from each treatment, villi and crypts were analysed from each sample at 42 days, and the average values were calculated for statistical analysis.

Real-time PCR to Access Nutrient Transporters Genes Expression

The expression of glucose transporters genes of (GLUT1, SGLT4, and GLUT5 and amino acids transporter genes (PEPT2, CAT1, and LAT1) were determined using RT-PCR. The mRNA was extracted from the intestinal mucosal sample of duodenum, jejunum, and ileum (*n*=10) per treatment using Trizol reagent (TaKaRa Biotechnology Co. Ltd., Dalian, Liaoning, China). The extracted RNA was treated with the RNeasy Mini kit, Qiagen according to the manufacturer's instructions. The quantity and purity of the total RNA were determined by a Nanodrop ND-

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8000 spectrophotometer (Thermo fisher Scientific, Waltham, USA). The complementary DNA (cDNA) was obtained by Reverse-transcription of the isolated RNA sample using Takara cDNA transfer kit (Bio-Rad, Hercules, CA). The PCR reaction mixture consists of twenty-five (25µL) containing 2.5 µL of the genomic DNA, 12.5 µL Master mix, 9 µL Nuclease Free water, and 0.5 µL of each reverse and forward primers. The reactions were run on a Master cycler Gradient 5331 thermal cycler with Initial denaturation at 94˚C for 5 min, followed by 35 cycles consisting of denaturation at 94˚C for 30 s, annealing at 55˚C for 1 min, extension at 72˚C for 1 min and a final extension at 72˚C for 2 min. β-actin was used to normalise the expression of the target genes, according to Livak and Schimittgen (2001). The primers used in this study were mostly sourced from previously published studies in chickens or otherwise designed using NCBI primer tool (https://www.ncbi.nlm.nih.gov/). (Table 2) shows the primers used in this study.

Table 2. Primer sequencing and target genes used for quantitative real-time PCR.

Gene symbol	Sequence (5′–3′)	Full Name	GenBank accession No.
GLUT1	F-TCCTCCTGATCAACCGCAAT R-TGTGCCCCGGAGCTTCT	Glucose transporter-1 (SLC7A1)	NM_205209.1
GLUT5	F-TTGCTGGCTTTGGGTTGTG RGGAGGTTGAGGGCCAAAGTC	Glucose transporter-5 (SLC2A5)	XM_417596
SGLT4	F-ATACCCAAGGTAATAGTCCCAAAC R- TGGGTCCCTGAACAAATGAAA	Sodium glucose transporter-4 (SLC2A4)	XM_04067852 1.2
PEPT2	F-TGACTGGGCATCGGAACAA R-ACCCGTGTCACCATTTTAACCT	Peptide transporter-2 (SLC15A2)	NM_00131902 8.1
CAT1	F-CAAGAGGAAAACTCCAGTAATTGCA R- AAGTCGAAGAGGAAGGCCATAA	Cationic amino acids transporter-1 (SLC7A1)	XM_01527794 5.1
LAT1	F-GATTGCAACGGGTGATGTGA R- CCCCACACCCACTTTTGTTT	L type amino acids transporter-1 (SLC7A5)	KT876067.1
β-actin	F-ATTGTCCACCGCAAATGCTTC R-AAATAAAGCCATGCCAATCTCGTC		NM_205518.1

[1]F: Forward, R: Reverse.

Statistical analysis

The experimental data were analysed using the GLM procedure of SAS (SAS Institute Inc. Base SAS®9.4, 2013). Post hoc Duncan test was carried out with SPSS to compare the means. Data were expressed as mean ±SEM. Treatments were considered significantly different at ($p < 0.05$.)

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RESULTS AND DISCUSSION

Growth performance

The growth performance of broilers fed a diet containing antibiotics and phytobiotics are shown in Table 2. In the first three weeks of the trial, chicken recorded similar body weight gain and FCR without any statistically significant difference (P> 0.05) except feed intake that was statistically significant (P=0.0001) higher in AGP-fed chicken compared with phytobiotic and CON groups. The average daily gain was statistically significant (P=0.0413) higher in the Phytobiotic fed diet than the AGP fed group, while the lowest gain was obtained in CON fed group 1-42d of age Table 2. Similarly, at 22-42days chickens fed phytobiotic had significantly (P < 0.05) lowest feed intake (P=0.0175) and had significantly (P< 0.05) lower feed conversion ratio in chicken fed with phytobiotic than the AGP and CON fed groups with no antibiotic or phytobiotic seen Table 2. These results obtained in this study were in agreement with that of Qamar et al. (2015), who obtained the highest body mass in broilers fed different phytobiotics through drinking water. Equally, Puvača et al., (2020) reported higher body mass on broiler chicken fed a diet containing chilli pepper and garlic powder than the treatment control without supplementation. Similar observations concerning the usage of natural supplements in broiler nutrition were noticed when used as natural growth promoters (Abd El-Ghany, 2020; Abd El-Hack et al., 2020; Ogbuewu et al., 2020). Similarly, (Akylidiz & Denli 2016) reported that the addition of phytobiotics of turmeric powder and garlic to chickens' diet has improved broiler growth and feed conversion ratio and decreased mortality rate. This may be associated with the presence of various important alkaloids with excellent biological properties that have a positive effect on the performance of broilers (Abudabos et al., 2016). On the contrary. (Erdogan et al., 2010 and Abdulkarim et al., 2013) did not find any significant effects on broiler growth performance fed a diet containing phytogenic compounds. However, FCR was significantly improved.

Table 2. Effect of phytobiotics and Antibiotic on growth performance of broiler Chickens

		Treatments					
Age		Parameters	CON	AGP	PB	SEM	*p-value*
1-21days		ADFI (g)	38.70^{b}	41.13^{a}	39.36^{b}	0.69	0.0001
		ADG (g)	32.71	36.85	32.96	1.83	0.3269
		FCR (g-g)	1.20	1.15	1.20	0.07	0.1634
22-42vdays		ADFI (g)	178.43^{ab}	186.72^{a}	154.60^{c}	9.69	0.0175
		ADG (g)	99.55	100.94	106.67	3.76	0.2590
		FCR (g-g)	1.79^{a}	1.85^{a}	1.45^{b}	0.06	0.0001
1-42days		ADFI (g)	108.89^{a}	113.92^{a}	101.65^{b}	5.26	0.0058
		ADG (g)	66.26^{b}	68.00^{ab}	70.69^{a}	1.97	0.0413
		FCR (g-g)	1.64^{a}	1.66^{a}	1.44^{b}	0.06	0.0001

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Note. ADFI: Average daily feed intake, ADG: average daily gain, FCR: feed conversion ratio. CON: control group chicken fed (basal diet, only with no AGP and no phytobiotics); AGP: antibiotic group (basal diet supplemented with 5 mg/kg tetramycin); PB: phytobiotic group (basal diet supplemented with 0.5g/kg of phytobiotics. [a-b]Means with the same row carrying different superscripts are significantly different ($p < 0.05$). Data were presented as means ± SE

Intestinal histomorphology

Results on the effect of antibiotics and phytobiotics on intestinal morphology from the duodenum, jejunum, and ileum of broiler chickens are presented in Table 3. Based on the results obtained, it was observed that both AGP and phytobiotic indicated statistically significant ($P < 0.05$) effects on villus height and crypt depth in duodenum, villi are longer in phytobiotic than in AGP-fed chickens while the lowest values were obtained in CON fed chickens, whereas the VH: CD are statistically significant ($P < 0.05$) higher in Phytobiotic fed chickens and similar in AGP and CON treatment fed broilers. In jejunum, the results of villus height, crypt depth, and VH: CD show statistically significant ($P < 0.05$) AGP and Phytobiotic fed chickens recorded the highest value of villus height and the lowest value were recorded in CON-fed chickens. In contrast, crypt depth was statistically significant ($P < 0.05$) higher in AGP fed groups than in phytobiotic and the lowest value were seen on CON fed treatment chickens, the VH:CD are statistically significant ($P < 0.05$) higher in phytobiotic and CON treatment groups than and the lowest ratio are recorded in AGP fed chickens.

Table 3. Effect of Phytobiotics and Antibiotics on Intestinal Histomorphology of Broiler Chickens

		Treatments				
Gut region	Parameters(µm)	CON	AGP	PB	SEM	*P-value*
Duodenum	Villus height	2295.3^{c}	2890.1^{b}	3548.3^{a}	27.26	0.0001
	Crypt depth	298.7^{b}	350.7^{a}	371.5^{a}	15.62	0.0001
	VH:CD	7.85^{b}	8.31^{b}	9.64^{a}	0.42	0.0003
Jejunum	Villus height	2439.6^{b}	2881.5^{a}	3069.2^{a}	1.04	0.0001
	Crypt depth	256.2^{c}	377.5^{a}	334.9^{b}	13.92	0.0001
	VH:CD	9.61^{a}	7.65^{b}	9.32^{a}	0.39	0.0001
Ileum	Villus height	1352.1^{c}	1505.1^{b}	1630.5^{a}	51.59	0.0001
	Crypt depth	268.00^{b}	280.7^{ab}	323.9^{a}	22.23	0.0399
	VH:CD	5.26	5.55	5.18	0.4	0.6265

Note. VH: CD: villus height to crypt depth ratio. CON: control group chicken fed (basal diet, only with no AGP no Phytobiotics); AGP antibiotic group (basal diet supplemented with 5 mg/kg tetramycin); PB: phytobiotic group ((basal diet supplemented with 0.5g/kg of phytobiotics. At 2 days of age, [a-c]Means within the same row carrying different superscripts were significantly different ($p < 0.05$). Data were presented as means ± SE

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In ileum, treatment groups indicated statistical significance ($P < 0.05$) on villus height and crypt depth, where phytobiotic fed groups exhibited statistically significantly ($P < 0.05$) higher values than of villus height than in AGP fed group while the lowest values were recorded in CON fed group, whereas, the crypt depth was higher in phytobiotic than in CON fed chickens, no statistical significant ($P > 0.05$) was detected in the VH: CD in the ileum of the boiler chickens. The results in this study was in accordance with the finding of (Dialoke et al., 2020) where the use of phytobiotics chestnut (*Castenea sativa*) on broiler birds exhibited significantly higher Villi area, perimeter, height, width and villi height/crypt depth ratio than that of birds fed AGP (Oxytetracycline) and control treatment chickens. The intestinal villi and crypt has been associated with intestinal functions and growth in chickens and higher intestinal villi are associated with increase absorptive surface area of the intestine and hence, an increase absorptive capacity with resultant higher body weight gain (Kanduri et al., 2013). Similarly, (Kavitha et al., 2023). Reported significant increase in villus height and Villus height and Crypt depth ratio on broilers fed phytobiotics mixture containing (*Syzygium aromaticum* buds + *Mentha spicata* leaves + *Emblica officinalis)* fruits) and (*Emblica officinalis* fruits + *Murraya kenigii* leaves + *Syzygium aromaticum* buds) respectively. Increase intestinal villi height and villi height to crypt depth is an indication of a large area for nutrients absorption and higher absorption efficiency (Sieo et al., 2005). Higher villi height, low crypt depth, and high villi height /crypt depth ratio are desirable parameters for better nutrient absorption (Xu et al., 2003).

The nutrients transporters mRNA genes expression

The mRNA expression of GLUT1, SGLT4, and GLUT5 was presented in (Figure 1). The findings indicated statistically significant ($P < 0.05$) up-regulation of GLUT1, SGLT4, and GLUT5 in duodenum and ileum in AGP and PB-fed chickens than in CON-fed chickens, while the amino acids nutrients transporters mRNA genes expression of PEPT2 is statistically significant ($P < 0.05$) higher expression and comparable in AGP and PB fed chickens was observed from jejunum and ileum compared to CON fed chickens while LAT1 was highly express by PB compared to the expression in AGP and CON fed chickens in the jejunum and ileum and CAT1 was significant ($P < 0.05$) up-regulation by AGP fed chickens from the duodenum and ileum as compared with the expression in chickens fed PB and CON diets Figure 2. The increased nutrient supply for growth and other reproductive functions is reflected in enhanced nutrient transport in the blood. These results indicated that chickens fed diets supplemented with either phytobiotics or AGP have significantly higher mRNA expression of nutrient transporters genes of glucose and amino acids, as mentioned above. Similar results were obtained by (Abdulkarim et al., 2013), where birds fed a diet containing a mixture of essential oils with *thymol* and *anethole* as leading active substances in chickens showed enhanced nutrient supply and its transport. Phytobiotics were reported to improve broiler intestinal status and subsequently promote growth (Aljumah et al., 2020). Similar evidence also suggests that herbs, spices, and various plant extracts have appetising, and digestion-stimulating properties (Diniz et al., 2020) and enhancement of the digestibility of nutrients (Hernandez et al., 2004). According to (Wenk, 2003), birds fed dietary plant extracts strongly stimulate the endocrine system and indirect metabolism and absorption of nutrients, and the greater expression of transporter genes will lead to a higher flood of nutrients into the intestinal cells and subsequently into the blood (Ruhnke et al., 2015).

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CONCLUSION

In the present study, a phytobiotics 0.5g/kg, Nbiotic™, the compound was tested alongside AGP to determine their efficacy on broiler growth performance, intestinal histomorphology, and nutrient transporters mRNA gene expression. The findings indicated that phytobiotics supplementation in broiler diets as feed additives recorded significantly(P < 0.05) highest body weight gain, Significantly (P< 0.05) recorded lower feed conversion ratio, increased Villus height, Crypt depth, and Villus height to Crypt depth Ratio. Likewise, the inclusion of phytobiotics in the diets of broilers showed Significantly (P < 0.05) higher mRNA expression of nutrients transporters genes of GLUT1, SGLT4, GLUT5, PEPT2, CAT1, and LAT1, which increase nutrients digestibility and its uptake, in this findings phytobiotics and AGP are almost correlative in their effects with one another when compared with the Control group without either of the supplementation. Therefore, these phytobiotics could potentially be used as natural growth promoters in broiler chickens and can substitute synthetic growth-promoting antibiotics. Moreover, a detailed investigation should be conducted to determine other effects and mechanisms of action of these phytobiotics and their safety on the broiler diet.

Conflict of Interest

The authors declare no conflict of interest.

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APPENDIX

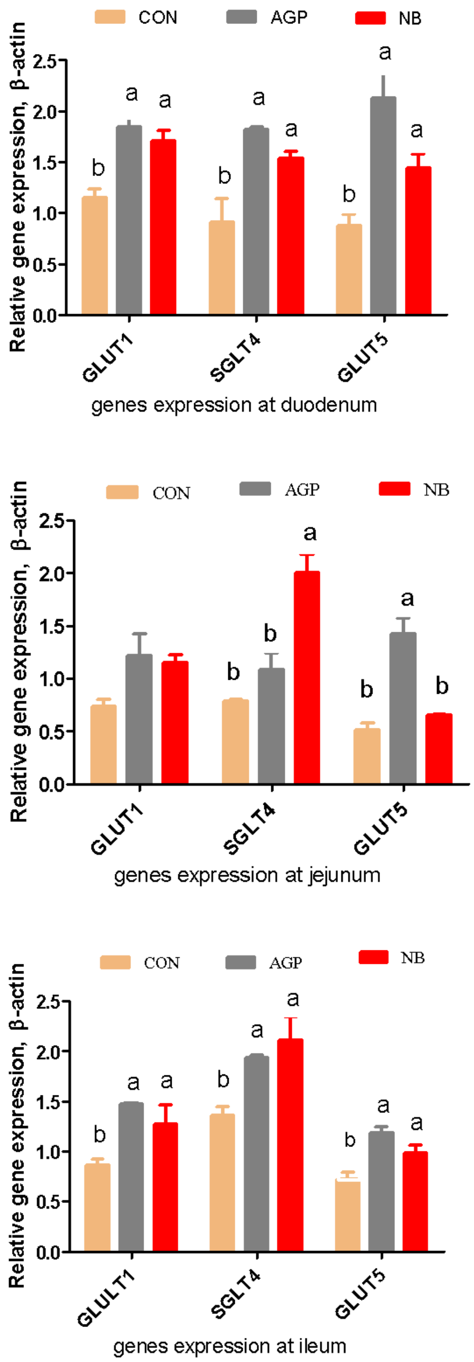

Figure1. Effects of Phytobiotics and AGP on glucose transporters mRNA genes expression of glucose transporter-1 (GLUT-1), Sodium dependent glucose transporter-4 (SGLT-4) and glucose transporter-5 (GLUT-5) at (a) duodenum (b) jejunum and (c) ileum of broilers at 42 days of age**;** CON: control group chicken fed (basal diet, only with no AGP no Phytobiotics); AGP antibiotic group chicken fed (basal diet supplemented with 5 mg/kg tetramycin); PB: phytobiotic group chicken fed (basal diet supplemented with 0.5g/kg of phytobiotics. [a-b]Means within the same column carrying different superscripts are significantly different at ($p < 0.05$). Data were presented as means ± SE

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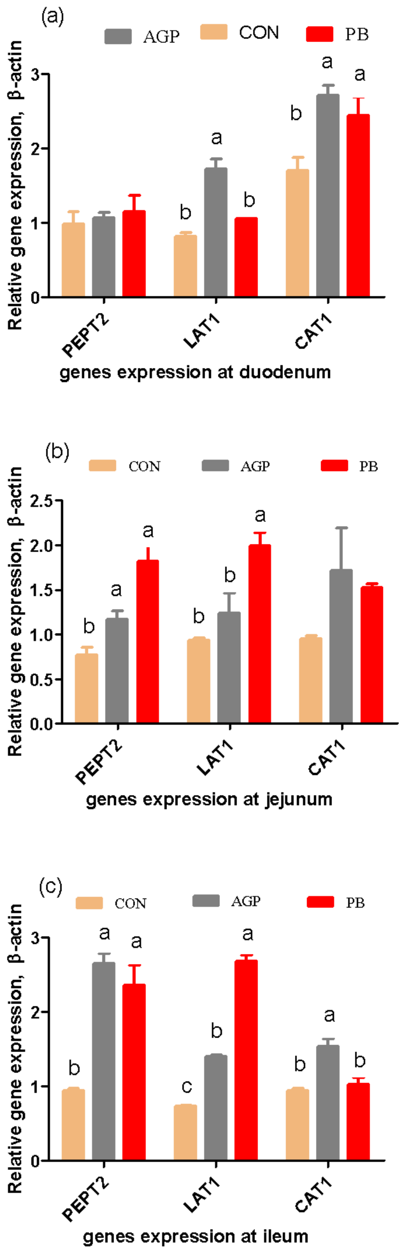

Figure2. Effects of Phytobiotics and AGP on amino acids mRNA genes expression of peptides transporter-2 (PEPT-2), L type amino acids transporter-1 (LAT-1) and Cationic amino acids transporter-1(CAT-1) at (a) duodenum (b) jejunum and (c) ileum of broilers at 42 days of age**;** CON: control group chicken fed (basal diet, only with no AGP no Phytobiotics); AGP antibiotic group chicken fed (basal diet supplemented with 5 mg/kg tetramycin); PB: phytobiotic group chicken fed (basal diet supplemented with 0.5g/kg of phytobiotics. [a-b]Means within the same column carrying different superscripts are significantly different at ($p < 0.05$).
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